REMARKS/ARGUMENTS

Claims 12, 13 and 15-17 are active.

Claims 12 and 13 are amended for clarity.

Claim 17 is amended to recite the production of a recombinant polypeptide using the host cell containing the isolated polynucleotide.

No new matter is added.

The rejection of Claims 14 and 18-19 under 35 USC 102(b) or 103(a) citing US 6,010,722 to Matsumoto *et al* is no longer applicable as those claims have been cancelled. Similarly, the rejection of Claim 14 under 35 USC 112, second paragraph is no longer applicable as Claim 14 has been cancelled.

The rejection of Claim 13 under 35 USC 102(b) or 103(a) citing Vuorio in view of Young, Nah, Sandell 1, Sandell 2 and Upholt is believed to be no longer applicable in light of the amendment submitted to Claim 13 in this paper. That is, when raising the rejection, the Examiner interpreted the claims to permit the application of any dinucleotide within SEQ ID NO:2 as anticipating Claim 13 and "all of the cited references encode a portion of type II chicken collagen." Page 8 of the Official Action. Claim 13 as amended in this paper recites "An isolated polynucleotide, comprising SEQ ID NO: 2" which means that the polynucleotide has at least all of the sequence defined in SEQ ID NO:2. See, e.g. MPEP 2111.03. Thus, the claim does not read on dinucleotides or portions of SEQ ID NO:2 but rather at least the entire sequence recited therein.

Withdrawal of the rejection is requested.

The rejection of Claims 15-17 under 35 USC 103(a) citing Vuorio, Young, Na, Sandell 1, Sandell 2, Upholt and Matsumoto is believed to be inapplicable for the same reason that the rejection of Claim 13 is overcome as discussed in the immediately preceding paragraphs. See also the Examiner's conclusion on page 11, first full paragraph relying on the claim interpretation of the earlier 103(a) rejection. Further, none of the cited documents describe or suggest the cloning of a polynucleotide sequence as defined in Claims 12 or 13. Claims 15 and 17 depend from Claims 12 or 13 and as a result incorporate all of the requirements that Claims 12 and 13 recite.

Withdrawal of the rejection is requested.

The rejection of Claim 17 under 35 USC 112, first paragraph is believed to be no longer applicable in light of the amended Claim 17 reciting the production of a recombinant polypeptide using a host cell including the isolated polynucleotides. That is, the Examiner (on page 14) alleges that the protein encoded by those sequences are not chicken type II collagen. However, the claim now reads the production of a polypeptide from the polynucleotide, something that is certainly well-enabled in the art.

Withdrawal of the rejection is requested.

The rejections applied to Claims 12, 15 and 16 under 35 USC 101 and 35 USC 112, first paragraph alleging that the claims lack a specific and substantial utility and as a result are not enabled. The rejection is based on the supposition that SEQ ID NO:1 does not encode a type II chicken collagen (see page 19, lines 1-2 of the Action).

Applicants respectfully disagree.

The full length cDNA sequence encoding chicken type II collagen is successfully cloned for the first time in the present application and successfully registered in US NCBI-

GenBank with an accession number of AY046949 version AY046949.1 GI: 15546069), and was published on February 8, 2006, wherein the AY046949 represents the version of mRNA of a chicken type II collagen protein and the accession number AAK98621 represents the amino acid sequence of the relevant chicken type II collagen protein of the present application that is obtained by the translation of the mRNA of the chicken type II collagen protein.

The applicant also published an article in the journal "Gene" (see, Xi C, Liu N, Liang F, GuoSQ, Sun YY, Yang FT, Xi YZ. Molecular cloning, characterization and localization of chicken type II procollagen gene. Gene, 2006, 366:67-76).

In the present application, the base sequence of the full length cDNA encoding a chicken type II collagen is explicitly recited in the sequence listing (see, SEQ ID NO: 1) in the sequence listing, and biological homological comparison is made between the full length cDNA and amino acid sequence of the chicken type II collagen protein CCOL2A1 and the gene sequences and amino acid sequences of chicken type II collagens in different species such as human, canine, zebrafish and mouse, and , which clearly demonstrates that the base sequence of the full length cDNA encoding a chicken type II collagen cloned in the present application is unique (see, the chicken CCOL2A1 homological comparison in Example 6 of the present application). Moreover, the corresponding research result is published in the journal "Gene" (see, Xi C, Liu N, Liang F, GuoSQ, Sun YY, Yang FT, Xi YZ. Molecular cloning, characterization and localization of chicken type II procollagen gene. Gene, 2006, 366:67-76), and this paper disclosed the following contents (see, page 71 paragraph 1 and page 72 paragraph 4), "The deduced amino acid sequence of the ccol2a1 gene in triple helix domain was aligned with the available counterparts of the human (NM-001844), mouse (M65161), canine (AF023169), rat (L48440), horse (U62528), frog (BC048221) and newt (AB022046). A phylogenetic tree was then constructed using the coding sequence of each

cDNA using distances between all pairs. The interspecies homologous comparison of the ccol2a1 with its counterparts in human, mouse, canine, horse, rat, frog and newt revealed that collagen type II is highly sequence identities in triple helical domain. The highest conservation was seen between chicken and canine, the identity is 94.77%, followed by the horse col2a1 (94%) and to human, mouse, newt and rat were 93%, 92%, 92%, 92% respectively, by which a phylogenetic tree of the ccol2a1 was constructed (Fig. 1-3). The result shows that the entire COL2A1 clustered together as a group".

```
    *         20         *         40         *         60
human   : GPMGPMGPRGPPGPAGAPGPQGFQGNPGEPGEPGVSGPMGPRGPPGPPGKPGDDGEAGKP :
60
horse   : GPMGPMGPRGPPGPAGAPGPQGFQGNPGEPGEPGVSGPMGPRGPPGPPGKPGDDGEAGKP :
60
mouse   : GPMGPMGPRGPPGPAGAPGPQGFQGNPGEPGEPGVSGPMGPRGPPGPAGKPGDDGEAGKP :
60
canine  : GPMGPMGPRGPPGPAGAPGPQGFQGNPGEPGEPGVSGPMGPRGPPGPPGKPGDDGEAGKP :
60
rat     : GPMGPMGPRGPPGPAGAPGPQGFQGNPGEPGEPGVSGPIGPRGPPGPAGKPGDDGEAGKP :
60
chicken : GPMGPMGPRGPPGPTGAPGPQGFQGNPGEPGEPGAAGPMGPRGPPGPPGKPGDDGETGKP :
60
frog    : GPMGPMGPRGPPGPTGAPGPQGFQGNPGEPGEPGAGGPMGPRGPPGPSGKPGDDGEAGKP :
60
newt    : GPMGPMGPRGPPGPSGSPGPQGFQGNPGEPGEPGAAGPMGPSGPPGPDGKPGDDGEQGKP :
60
          GPMGPMGPRGPPGP GaPGPQGFQGNPGEPGEPG  GP6GPrGPPGP GKPGDDGEaGKP

                 *        80         *        100        *        120
human   : GKAGERGPPGPQGARGFPGTPGLPGVKGHRGYPGLDGAKGEAGAPGVKGESGSPGENGSP :
120
horse   : GKSGERGPPGPQGARGFPGTPGLPGVKGHRGYPGLDGAKGEAGAPGVKGESGSPGENGSP :
120
mouse   : GKSGERGLPGPMGARGFPGTPGLPGVKGHRGYPGLDGAKGEAGAPGVKGESGSPGENGSP :
120
canine  : GKSGERGPPGPQGARGFPGTPGLPGVKGHRGYPGLDGAKGEAGAPGVKGESGSPGENGSP :
120
rat     : GKAGERGLPGPQGARGFPGTPGLPGVKGHRGYPGLDGAKGEAGAPGVKGESGSPGENGSP :
120
chicken : GKSGERGPPGPQGARGFPGTPGLPGVKGHRGYPGLDGAKGEAGAPGAKGESGSPGENGSP :
120
frog    : GKSGERGPPGPQGARGFPGTPGLPGVKGHRGYPGLDGAKGEAGAAGAKGEGGATGEAGSP :
120
newt    : GKNGERGPPGPQGARGFPGTPGLPGVKGHRGYPGLDGAKGEAGAAGAKGEGGAPGENGAP :
120
          GK GERGpPGPqGARGFPGTPGLPGVKGHRGYPGLDGAKGEAGApG KGEsGspGEnGsP

                 *       140         *        160        *        180
human   : GPMGPRGLPGERGRTGPAGAAGARGNDGQPGPAGPPGPVGPAGGPGFPGAPGAKGEAGPT :
180
horse   : GPMGPRGLPGERGRTGPAGAAGARGNDGQPGPAGPPGPVGPAGGPGFPGAPGAKGEAGPT :
180
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mouse   : GPMGPRGLPGERGRTGPAGAAGARGNDGQPGPAGPPGPVGPAGGPGFPGAPGAKGEAGPT : 180
canine  : GPMGPRGLPGERGRTGPAGAAGARGNDGQPGPAGPPGPVGPAGGPGFPGAPGAKGEAGPT : 180
rat     : GPMGPRGLPGERGRTGPAGAAGARGNDGQPGPAGPPGPVGPAGGPGFLGAPGAKGEAGPT : 180
chicken : GPMGPRGLPGERGRPGPSGAAGARGNDGLPGPAGPPGPVGPAGAPGFPGAPGSKGEAGPT : 180
frog    : GPMGPRGLPGERGRPGSSGAAGARGNDGLPGPAGPPGPVGPAGAPGFPGAPGSKGEAGPT : 180
newt    : GPMGPRGLPGERGRPGPSGAAGARGNDGLPGPAGPPGPVGPAGAPGFPGAPGSKGEAGPT : 180
          GPMGPRGLPGERGR Gp GAAGARGNDG PGPAGPPGPVGPAG PGFpGAPG KGEAGPT

                      *      200         *        220        *       240
human   : GARGPEGAQGPRGEPGTPGSPGPAGASGNPGTDGIPGAKGSAGAPGIAGAPGFPGPRGPP : 240
horse   : GARGPEGAQGPRGEPGTPGSPGPAGAAGNPGTDGIPGAKGSAGAPGIAGAPGFPGPRGPP : 240
mouse   : GARGPEGAQGSRGEPGNPGSPGPAGASGNPGTDGIPGAKGSAGAPGIAGAPGFPGPRGPP : 240
canine  : GARGPEGAQGPRGEPGTPGSPGPAGASGNPGTDGIPGAKGSAGAPGIAGAPGFPGPRGPP : 240
rat     : GARGPEGAQGSRGEPGNPGSPGPAGASGNPGTDGIPGAKGSAGAPGIAGAPGFPGPRGPP : 240
chicken : GARGPEGAQGPRGESGTPGSPGPAGAPGNPGTDGIPGAKGSAGAPGIAGAPGFPGPRGPP : 240
frog    : GARGPEGAQGPRGESGTPGSPGPAGASGNPGTDGIPGAKGSSGGPGIAGAPGFPGPRGPP : 240
newt    : GARGPEGPQGPRGESGTPGSPGPSGASGNPGTDGIPGAKGSAGAPGIAGAPGFPGPRGPP : 240
          GARGPEGaQGpRGE GtPGSPGPaGAsGNPGTDGIPGAKGSaGaPGIAGAPGFPGPRGPP

                      *      260         *        280        *       300
human   : GPQGATGPLGPKGQTGEPGIAGFKGEQGPKGEPGPAGPQGAPGPAGEEGKRGARGEPGGV : 300
horse   : GPQGATGPLGPKGQTGEPGIAGFKGEQGPKGEPGPAGPQGAPGPAGEEGKRGARGEPGGA : 300
mouse   : GPQGATGPLGPKGQAGEPGIAGFKGDQGPKGETGPAGPQGAPGPAGEEGKRGARGEPGGA : 300
canine  : GPQGATGPLGPKGQTGEPGIAGFKGEQGPKGEPGPAGPQGAPGPAGEEGKRGARGEPGGA : 300
rat     : GPQGATGPLGPKGQTGEPGIAGFKGEQGPKGETGPAGPQGAPGPAGEEGKRGARGEPGGA : 300
chicken : GPQGATGPLGPKGQTGEPGIAGFKGEQGPKGETGPAGPQGAPGPAGEEGKRGARGEPGAA : 300
frog    : GPQGATGPLGPKGQTGDPGVAGFKGEQGPKGEIGSAGPQGAPGPAGEEGKRGARGEPGAA : 300
newt    : GPQGATGPLGPKGQTGDPGVAGFKGEQGPKGEIGPSGPQGAPGPAGEEGKRGARGEPGAA : 300
          GPQGATGPLGPKGQtGePG6AGFKGeQGPKGE GpaGPQGAPGPAGEEGKRGARGEPG a

                      *      320         *        340        *       360
human   : GPIGPPGERGAPGNRGFPGQDGLAGPKGAPGERGPSGLAGPKGANGDPGRPGEPGLPGAR : 360
horse   : GPVGPPGERGAPGNRGFPGQDGLAGPKGAPGERGPSGLAGPKGANGDPGRPGEPGLPGAR : 360
mouse   : GPIGPPGERGAPGNRGFPGQDGLAGPKGAPGERGPSGLAGPKGANGDPGRPGEPGLPGAR : 360
canine  : GPVGPPGERGAPGNRGFPGQDGLAGPKGAPGERGPSGLAGPKGANGDPGRPGEPGLPGAR : 360
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rat     : GPIGPPGERGAPGNRGFPGQDGLAGPKGAPGERGPSGLAGPKGANGDPGRPGEPGLPGAR :
360
chicken : GPVGPPGERGAPGNRGFPGQDGLAGPKGAPGERGPAGLAGPKGATGDPGRPGEPGLPGAR :
360
frog    : GPNGPPGERGAPGNRGFPGQDGLAGPKGAPGERGVPGLGGPKGGNGDPGRPGEPGLPGAR :
360
newt    : GPLGPNGERGAPGNRGFPGQDGLPGPKGAPGERGVAGLGGPKGANGDPGRPGEPGLPGVR :
360
          GP GPpGERGAPGNRGFPGQDGLaGPKGAPGERGp GLaGPKGanGDPGRPGEPGLPGaR

                    *        380         *        400         *        420
human   : GLTGRPGDAGPQGKVGPSGAPGEDGRPGPPGPQGARGQPGVMGFPGPKGANGEPGKAGEK :
420
horse   : GLTGRPGDAGPQGKVGPSGAPGEDGRPGPPGPQGARGQPGVMGFPGPKGANGEPGKAGEK :
420
mouse   : GLTGRPGDAGPQGKVGPSGAPGEDGRPGPPGPQGARGQPGVMGFPGPKGANGEPGKAGEK :
420
canine  : GLTGRPGDAGPQGKVGPSGAPGEDGRPGPPGPQGARGQPGVMGFPGPKGANGEPGKAGEK :
420
rat     : GLTGRPGDAGPQGKVGPSGAPGEDGRPGPPGPQGARGQPGVMGFPGPKGANGEPGKAGEK :
420
chicken : GLTGRPGDAGPQGKVGPTGAPGEDGRPGPPGPQGARGQPGVMGFPGPKGANGEPGKAGEK :
420
frog    : GLTGRPGDAGPQGKVGPSGASGEDGRPGPPGPQGARGQPGVMGFPGPKGANGEPGKAGEK :
420
newt    : GLTGHPGDAGPQGKVGPTGAAGEDGRPGPPGPQGARGQPGVMGFPGPKGANGEPGKGGEK :
420
          GLTGrPGDAGPQGKVGP3GApGEDGRPGPPGPQGARGQPGVMGFPGPKGANGEPGKaGEK

                    *        440         *        460         *        480
human   : GLPGAPGLRGLPGKDGETGAAGPPGPAGPAGERGEQGAPGPSGFQGLPGPPGPPGEGGKP :
480
horse   : GLPGAPGLRGLPGKDGETGAAGPPGPAGPAGERGEQGAPGPSGFQGLPGPPGPPGEGGKP :
480
mouse   : GLAGAPGLRGLPGKDGETGAAGPPGPSGPAGERGEQGAPGPSGFQGLPGPPGPPGEGGKQ :
480
canine  : GLPGAPGLRGLPGKDGETGAAGPPGPAGPAGERGEQGAPGPSGFQGLPGPPGPPGEGGKP :
480
rat     : GLAGAPGLRGLPGKDGETGAAGPPGPSGPAGERGEQGAPGPSGFQGLPGPPGPPGEGGKQ :
480
chicken : GLPGAPGLRGLPGKDGETGAAGPPGPAGPVGERGEQGAPGPSGFQGLPGPPGPPGESGKP :
480
frog    : GLVGAPGLRGLPGKDGETGSQGPNGPAGPAGERGEQGPPGPSGFQGLPGPPGSPGEGGKP :
480
newt    : GLAGAPGLRGLSGKDGETGAQGPSGPAGPAGERGEQGPPGPNGFQGLPGPPGPPGEGGKP :
480
          GL GAPGLRGLpGKDGETGaaGPpGPaGPaGERGEQGaPGPsGFQGLPGPPGpPGEgGKp

                    *        500         *        520         *        540
human   : GDQGVPGEAGAPGLVGPRGERGFPGERGSPGAQGLQGPRGLPGTPGTDGPKGASGPAGPP :
540
horse   : GDQGVPGEAGAPGLVGPRGERGFPGERGSPGAQGLQGARGLPGTPGTDGPKGASGPAGPP :
540
mouse   : GDQGIPGEAGAPGLVGPRGERGFPGERGSPGAQGLQGPRGLPGTPGTDGPKGAAGPDGPP :
540
canine  : GDQGVPGEAGAPGLVGPRGERGFPGERGSPGAQGLQGPRGLPGTPGTDGPKGASGPAGPP :
540
rat     : GDQGIPGEAGAPGLVGPRGERGFPGERGSPGAQGLQGPRGLPGTPGTDGPKGAAGPDGPP :
540
chicken : GDQGVPGEAGAPGLVGPRGERGFPGERGSPGAQGLQGPRGLPGTPGTDGPKGATGPAGPN :
540
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frog : GDQGVPGEAGAPGLVGPRGERGFPGERGSSGPQGLQGPRGLPGTPGTDGPKGASGPSGPN : 540
newt : GDQGVPGEAGTPGLVGPRGERGFPGERGSSGPQGLQGPRGLPGTPGTDGPKGATGPSGPN : 540
GDQG6PGEAGaPGLVGPRGERGFPGERGSpGaQGLQGpRGLPGTPGTDGPKGA GP GP

* 560 * 580 * 600
human : GAQGPPGLQGMPGERGAAGIAGPKGDRGDVGEKGPEGAPGKDGGRGLTGPIGPPGPAGAN : 600
horse : GAQGPPGLQGMPGERGAAGIAGPKGDRGDVGEKGPEGAPGKDGGRGLTGPIGPPGPAGAN : 600
mouse : GAQGPPGLQGMPGERGAAGIAGPKGDRGDVGEKGPEGAPGKDGGRGLTGPIGPPGPAGAN : 600
canine : GAQGPPGLQGMPGERGAAGIAGPKGDRGDVGEKGPEGAPGKDGGRGLTGPIGPPGPAGAN : 600
rat : GAQGPPGLQGMPGERGAAGIAGPKGDRGDVGEKGPEGAPGKDGGRGLTGPIGPPGPAGAN : 600
chicken : GAQGPPGLQGMPGERGAAGIAGLKGDRGDVGEKGPEGAPGKDGARGLTGPIGPPGPAGPN : 600
frog : GAQGPPGLQGMPGERGAAGISGPKGDRGDTGEKGPEGASGKDGSRGLTGPIGPPGPAGPN : 600
newt : GAQGPPGLQGMPGERGTSGISGPKGDRGDVGEKGPEGASGKDGARGLTGPIGPPGPSGPN : 600
GAQGPPGLQGMPGERGaaGIaGpKGDRGDvGEKGPEGApGKDG RGLTGPIGPPGPaG N

* 620 * 640 * 660
human : GEKGEVGPPGPAGSAGARGAPGERGETGPPGPAGFAGPPGADGQPGAKGEQGEAGQKGDA : 660
horse : GEKGEVGPPGPAGTAGARGAPGERGETGPPGPAGFAGPPGADGQPGAKGEQGEAGQKGDA : 660
mouse : GEKGEAGPPGPSGSTGARGAPGEPGETGPPGPAGFAGPPGADGQPGAKGDQGEAGQKGDA : 660
canine : GEKGEVGPPGPAGTAGARGAPGERGETGPPGPAGFAGPPGADGQPGAKGEQGEAGQKGDA : 660
rat : GEKGEVGPPGPSGSTGARGAPGERGETGPPGPAGFAGPPGADGQPGAKGDQGEAGQKGDA : 660
chicken : GEKGESGPPGPSGAAGARGAPGERGEPGAPGPAGFAGPPGADGQPGAKGEQGEPGQKGDA : 660
frog : GEKGESGPSGPPGIVGARGAPGDRGENGPPGPAGFAGPPGADGQSGLKGDQGESGQKGDA : 660
newt : GEKGESGPPGPVGAVGARGAPGDRGESGAPGPAGFAGPPGADGQPGIKGEHGESGQKGDA : 660
GEKGE GPpGP G GARGAPGerGE GpPGPAGFAGPPGADGQpGaKG qGE GQKGDA

* 680 * 700 * 720
human : GAPGPQGPSGAPGPQGPTGVTGPKGARGAQGPPGATGFPGAAGRVGPPGSNGNPGPPGPP : 720
horse : GAPGPQGPSGAPGPQGPTGVTGPKGARGAQGPPGATGFPGAAGRVGPPGSNGNPGPPGPP : 720
mouse : GAPGPQGPSGAPGPQGPTGVTGPKGARGAQGPPGATGFPGAAGRVGPPGANGNPGPAGPP : 720
canine : GAPGPQGPSGAPGPQGPTGVTGPKGARGAQGPPGATGFPGAAGRVGPPGSNGNPGPPGPP : 720
rat : GAPGPQGPSGAPGPQGPTGVTGPKGARGAQGPPGATGFPGAAGRVGPPGSNGNPGPAGPP : 720
chicken : GAPGPQGPSGAPGPQGPTGVTGPKGARGAQGPPGATGFPGAAGRVGPPGPNGNPGPPGPP : 720
frog : GAPGPQGPSGAPGPQGPTGVFGPKGARGAQGPAGATGFPGAAGRVGTPGPNGNPGPPGPP : 720
newt : GAPGPQGPSGAPGPQGPTGVNGPKGARGAQGPPGATGFPGAAGRVGPPGPNGNPGAPGPP : 720

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           GAPGPQGPSGAPGPQGPTGVtGPKGARGAQGPpGATGFPGAAGRVGpPG NGNPGppGPP

                         *        740         *        760         *        780
human    : GPSGKDGPKGARGDSGPPGRAGEPGLQGPAGPPGEKGEPGDDGPSGAEGPPGPQGLAGQR :
780
horse    : GPSGKDGPKGARGDSGPPGRAGDPGLQGPAGPPGEKGEPGDDGPSGPDGPPGPQGLAGQR :
780
mouse    : GPAGKDGPKGVRGDSGPPGRAGDPGLEGPAGAPGEKGEPGDDGPSGLDGPPGPQGLAGQR :
780
canine   : GPSGKDGPKGARGDSGPPGRAGDPGLQGPAGPPGEKGEPGDDGPSGPDGPPGPQGLAGQR :
780
rat      : GPAGKDGPKGARGDTGAPGRAGDPGLQGPAGAPGEKGEPGDDGPSGSDGPPGPQGLAGQR :
780
chicken  : GSAGKDGPKGVRGDAGPPGRAGDPGLQGPAGPPGEKGEPGEDGPAGPDGPPGPQGLAGQR :
780
frog     : GSAGKEGPKGVRGDAGPPGRAGDPGLQGAAGAPGEKGEPGEDGPSGPDGPPGPQGLSGQR :
780
newt     : GSAGKDGPKGARGDGGPPGRAGDPGLQGPAGAPGEKGEPGEDGPNGPDGPPGPQGLAGQR :
780
           G   GKdGPKG RGD GpPGRAGdPGL2GpAG PGEKGEPG DGPsG dGPPGPQGLaGQR

                         *        800         *        820         *        840
human    : GIVGLPGQRGERGFPGLPGPSGEPGKQGAPGASGDRGPPGPVGPPGLTGPAGEPGRQGSP :
840
horse    : GIVGLPGQRGERGFPGLPGPSGEPGKQGAPGASGDRGPPGPVGPPGLTGPAGEPGREGTP :
840
mouse    : GIVGLPGQRGERGFPGLPGPSGEPGKQGAPGASGDRGPPGPVGPPGLTGPAGEPGREGSP :
840
canine   : GIVGLPGQRGERGFPGLPGPSGEPGKQGAPGASGDRGPPGPVGPPGLTGPSGEPGREGSP :
840
rat      : GIVGLPGQRGERGFPGLPGPSGEPGKQGAPGASGDRGPPGPVGPPGLTGPAGEPGREGSP :
840
chicken  : GIVGLPGQRGERGFPGLPGPSGEPGKQGAPGSAGDRGPPGPVGPPGLTGPAGEPGREGNP :
840
frog     : GIVGLPGQRGERGFPGLPGPSGEPGKQGGPGSSGDRGPPGPVGPPGLTGPSGEPGREGNP :
840
newt     : GIVGLPGQRGERGFPGLPGPSGEPGKQGSPGSAGDRGPPGPVGPPGLTGPAGEPGREGNP :
840
           GIVGLPGQRGERGFPGLPGPSGEPGKQGaPG sGDRGPPGPVGPPGLTGPaGEPGR2G P

                         *        860         *        880         *        900
human    : GADGPPGRDGAAGVKGDRGETGAVGAPGTPGPPGSPGPAGPTGKQGDRGEAGAQGPMGPS :
900
horse    : GADGPPGRDGAAGVKGDRGEAGALGAPGAPGPPGSPGPAGPTGKQGDRGEAGAQGPMGPA :
900
mouse    : GADGPPGRDGAAGVKGDRGETGALGAPGAPGPPGSPGPAGPTGKQGDRGEAGAQGPMGPS :
900
canine   : GADGPPGRDGAAGVKGDRGETGPVGAPGAPGSPGSPGPAGPTGKQGDRGEAGAQGPMGPA :
900
rat      : GADGPPGRDGAAGVKGDRGETGALGAPGAPGPPGSPGPAGPTGKQGDRGEAGAQGPMGPS :
900
chicken  : GADGLPGRDGAAGVKGDRGETGPVGAPGAPGAPGAPGPVGPTGKQGDRGETGAQGPMGPS :
900
frog     : GSDGPPGRDGATGIKGDRGETGPLGAPGAPGAPGAPGSVGPTGKQGDRGESGPQGPLGPS :
900
newt     : GSDGPPGRDGSLGVKGDRGETGPVGAPGAPGAPGSPGPVGPTGKQGDRGEAGPQGPLGPS :
900
           GaDGpPGRDGaaG6KGDRGEtG 6GAPGaPG PGsPGp GPTGKQGDRGEaGaQGP6GPs

                         *        920         *        940         *        960
human    : GPAGARGIQGPQGPRGDKGEAGEPGERGLKGHRGFTGLQGLPGPPGPSGDQGASGPAGPS :
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960
horse    : GPAGARGLPGPQGPRGDKGEAGEAGERGLKGHRGFTGLQGLPGPPGPSGDQGASGPAGPS :
960
mouse    : GPAGARGIAGPQGPRGDKGESGEQGERGLKGHRGFTGLQGLPGPPGPSGDQGASGPAGPS :
960
canine   : GPAGARGIPGPQGPRGDKGEAGEAGERGLKGHRGFTGLQGLPGPPGPSGDQGASGPAGPS :
960
rat      : GPAGARGIAGPQGPRGDKGEAGEPGERGLKGHRGFTGLQGLPGPPGPSGDQGTSGPAGPS :
960
chicken  : GPAGARGMPGPQGPRGDKGETGEAGERGLKGHRGFTGLQGLPGPPGPSGDQGAAGPAGPS :
960
frog     : GPAGARGLAGPQGPRGDKGEAGEAGERGQKGHRGFTGLQGLPGPPGSAGDQGATGPAGPA :
960
newt     : GPAGARGMPGPQGPRGDKGEAGEAGERGQKGHRGFTGLQGLPGPPGTAGDQGASGPSGPA :
960
           GPAGARG6 GPQGPRGDKGEaGE GERGlKGHRGFTGLQGLPGPPGpsGDQGa GPaGPs

                   *       980         *         1000        *
human    : GPRGPPGPVGPSGKDGANGIPGPIGPPGPRGRSGETGPAGPPGNPGPPGPPGPP : 1014
horse    : GPRGPPGPVGPSGKDGANGIPGPIGPPGPRGRSGETGPAGPPGNPGPPGPPGPP : 1014
mouse    : GPRGPPGPVGPSGKDGSNGIPGPIGPPGPRGRSGETGPVGPPGSPGPPGPPGPP : 1014
canine   : GPRGPPGPVGPSGKDGANGIPGPIGPPGPRGRSGETGPAGPPGNPGPPGPPGPP : 1014
rat      : GPRGPPGPVGPSGKDGSNGIPGPIGPPGPRGRSGETGPAGPPGNPGPPGPPGPP : 1014
chicken  : GPRGPPGPVGPSGKDGSNGMPGPIGPPGPRGRSGEPGPAGPPGNPGPPGPPGPP : 1014
frog     : GPRGPPGPVGPSGKDGSNGISGPIGPPGPRGRSGETGPSGPPGQPGPPGPPGPP : 1014
newt     : GPRGPPGPVGPSGKDGSNGLPGPIGPPGPRGRGGETGPAGPPGNPGPPGPPGPP : 1014
           GPRGPPGPVGPSGKDG NG6pGPIGPPGPRGRsGEtGPaGPPGnPGPPGPPGPP
```

Fig.1 Comparison of the deduced amino acid sequences of *ccol2a1* with seven counterparts. The sequence alignment was carried out with *DNASTAR* software. Amino acid sequences of *col2a1* from chicken (**AY046949**), human (**NM-001844**), mouse (**M65161**), canine (**AF023169**), rat (**L48440**), horse (**U62528**), frog (**BC048221**) and newt (**AB022046**) were aligned.

Percent Identity

Divergence	1	2	3	4	5		
1		94.8	98.5	96.2	84.3	1	ca3042.seq
2	5.4		93.9	92.9	87.4	2	ch3042.seq
3	1.5	6.4		96.2	82.7	3	h3042.seq
4	4.0	7.5	4.0		83.8	4	m3042.SEQ
5	17.7	13.8	19.7	18.3		5	zedna.seq
	1	2	3	4	5		

Fig 2. The homologous between human, canine, mouse, chicken and zebrafish upon the type II collagen sequence.

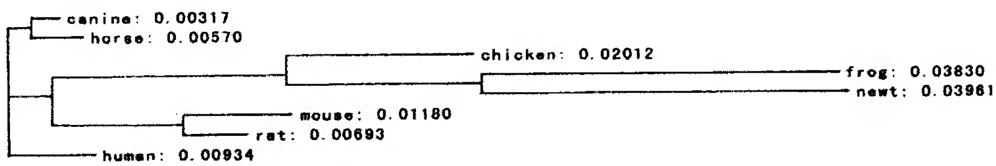

Fig.3. A phylogenetic tree for eight species of *col2a1*. The tree was generated using the clustal method with PAM250 residue weight table. The corresponding sequences included in this study are human *col2a1* (**NM-001844**), mouse *col2a1* (**M65161**), canine *col2a1* (**AF023169**), chick *col2a1* (**AY046949**), horse *col2a1* (**U62528**), rat *col2a1* (**L48440**), newt *col2a1* (**AB022046**) and frog *col2a1* (**BC048221**)

The present application provides Examples 7-11 demonstrating that the full length cDNA sequence can be used to effectively express a protein in various yeast vectors and identified by Western-Blotting analysis. Not only single expression of the cloned chicken type II collagen cDNA in various yeast vectors e.g. pPICZ αB/CCOL2A1 and pPIC9K/CCoL2A1 (Example 7), co-expression of the full length cDNA sequence and two subunits P4Hα,β of praline hydroxylase in yeast vectors e.g. pPIC9K/P4Hα, pPIC9/P4Hβ, and pPICZ α B/CCOL2A1 (Example 9), and the co-expression of pPIC9K/CCOL2A and pPICZ α A/P4Hα-β (Example 11). In addition, as to the α peptide chains of the expressed chicken type II collagens are subjected to Western-Blotting identification using a monoclonal antibody 95D1A specific to the α peptide chain of the collagen region.

The single expression of the chicken type II collagen cDNA is induced by BMGY or BMMMY medium in various yeast vectors such as pPICZαB/CCOL2A1 and pPIC9K/CCOL2A1. The supernatant and cytoplasmic fractions from the expressed product

are subjected to SDS-PAGE electrophoresis and Western blotting analysis using a monoclonal antibody 95D1 specific to the α peptide chain in collagen region. The results show that an 80 KD band rather than a 110 KD full length band representing the CCOL2A1α chain is only in the cytoplasm of the pPICZα B/CCOL2A1 transformant (see, the present invention, Example 7 and Figure 11A). This is because that when the pPICZαB vector is inserted into a full length CCOL2A1 exogenous gene, as the exogenous gene is relatively big, this gene certainly effects the startup of the vector, resulting in an incomplete expression of the full length target gene. In addition, it is possible that the α peptide chain of the expressed product chicken type II collagen is degraded in yeast and thus only an α peptide chain having a molecular weight of more than 80 KD is obtained. It has been shown that after the production of the hydroxylated intact peptide chain, about 10%-60% of a newly synthesized collagen is degraded before cell secretion, which modulates the quantity and quality of the collagen by cell.

In contrast, when a multi-copy expression vector pPICK/CCOL2A1 is employed, an α peptide of specific chicken type II collagen having a molecular weight of 110 KD is obtained (see, the present application, Example 7, and Figure 11B). If co-expression of pPIC9K/PH4α, pPIC9/P4Hβ and pPICZαB/CCOL2A1 is performed, the results show that the full length of CCOL2A1 chain is expressed in the cytolysate, but not in the supernatants (see, the present application, Figure 12).

A therapeutic vaccine that can be used to effectively treat rheumatoid arthritis is successfully prepared by using the full length cDNA encoding a chicken type II collagen CCOL2A1, and the relevant research result is published in the journal "Vaccines" (see, Song Xinqiang, Liang Fei, Liu Nan, Luo Yuan, Xue Hong, Yuan Fang, Tan Liuxin, Sun Yuying, Xi Caixi, Xi YongzhiConstruction and characterization of a novel DNA vaccine that is potent antigen-specific tolerizing therapy for experimental arthritis by increasing CD4+CD25+Treg

cells and inducing Th1 to Th2 shift in both cells and cytokines. Vaccine, 2009, 27 : 690-700).

The novel chicken pcDNA-CCOL2A1 therapeutic DNA vaccine prepared by using the full length cDNA encoding a chicken type II collagen CCOL2A1, SDS/PAGE, Western-blotting and ESI-MS/MS(electrospray ionization-tandem mass spectrometry) are employed to systematically analyze the expression of a secreted chicken type II collagen by the vaccine pcDNA-CCOL2A1 containing the full length cDNA encoding a chicken type II collagen CCOL2A1 in COS-7 cells. Further, Palladium-coated Borosilicate Electrospray Needle method is employed to analyze the sequence of the secreted chicken type II collagen expressed in COS-7 cells. The results show that the novel chicken pcDNA-CCOL2A1 therapeutic DNA vaccine prepared by using the full length cDNA encoding a chicken type II collagen CCOL2A1 can express a secreted chicken type II collagen in COS-7 cells, please see "Vaccines", 2009, 27 : 690-700, p693 paragraphs 3-6), reproduced below.

"Construction of pcDNA-CCOL2A1 tolerizing DNA vaccine

It has been well established that CCII has several specific biological functions. Importantly, CII made from other sources, such as cattle and sharks, does not produce the same superior results as joint relief. Recently, we have successfully cloned the full-length cDNA and nearly complete genomic DNA encoding CCOL2A1. All of these are both theoretical and material base for the development of a novel tolerizing DNA vaccine pcDNA-CCOL2A1.

To generate pcDNA-CCOL2A1 vaccine, we first PCR amplified the cDNA encoding the pC1 (II) chain procollagen (pC-procollagen) of CCII (CCOL2A1) from a plasmid previously constructed by our laboratory. Because the C-propeptide domains of the pro chains are essential for correct chain recognition and intracellular assembly of triple helices, while the

N-propeptide domains of the pro chains play little or no role, this product, the 4000 bp pC1(II), was designed to extended from the N-telopeptide sequence to the TAA termination codon of pC1(II) and flanked by KpnI restriction sites by which the N-propeptides of CCII could be deleted. The CCOL2A1 sequences were confirmed by DNA sequencing and then cloned into the pcDNA3.1(+) eukaryotic expression vector. The pcDNA-CCOL2A1 vaccine, with CCOL2A1 under control of the cytomegalovirus (CMV) promoter was constructed as described in figure 1A. To achieve the optimal expression of the target gene CCOL2A1, the signal peptide sequence and the Kozak consensus sequence were included before the ATG start codon. When the recombinant plasmid pcDNA-CCOL2A1 was cut with EcoR I and Hind III, the 4.3kb CCOL2A1 band and 5.4kb pcDNA3.1 band were observed, meanwhile when it was cut with Hind III, a about 9.7kb band was observed. From these results it can be concluded that recombinant pcDNA-CCOL2A1 vaccine was constructed successfully.

Expression and characterization of pcDNA-CCOL2A1 tolerizing DNA vaccine

Theoretically, the major recombinant expression systems, such as bacteria, yeast, and insect cells, now available for the production of proteins are unsuitable for expression of recombinant CCII, since they all lack sufficient prolyl-4-hydroxylase (P4H) and glycosylase activities. It is reasonable to assume that the lack of both hydroxylation and glycosylation should markedly affect the ability of CII epitopes to induce immune tolerance. Thus, we first examined the expression levels of pcDNA-CCOL2A1 in COS-7 cells that is especially suited for transient expression with recombinant plasmids. Supernatants from transfected COS-7 cells were analyzed by SDS/PAGE and western blotting with anti-chicken-CII mouse monoclonal antibody and a sheep anti-mouse peroxidase-conjugated antibody and then visualized by Coomassie brilliant blue staining and by enhanced chemiluminescence detection, respectively. We observed a band of approximately 120 kD, corresponding to the full-length CCII with deleted N-propeptides. No band was present in control samples

transfected with the empty vector negative control (pcDNA3.1). From these results, we can conclude that pcDNA-CCOL2A1 vaccine is able to properly express and export the α chains of CCII in COS-7 cells.

We next investigated whether the CCII protein produced in COS-7 cells is properly hydroxylated and glycosylated to ensure its therapeutic efficacy, as established in the rat CIA model. We used ESI-MS/MS (electrospray ionization-tandem mass spectrometry) to analyze the CII protein after "in gel" trypsin digestion. Peptide sequencing was performed using a palladium-coated borosilicate electrospray needle. The amino acid sequences of the peptides were deduced with the peptide sequencing program MasSeq. A database search was performed with the Mascot search engine (http://www.matrixscience.co.uk) using the data processed through MaxEnt3 and MasSeq. The partial peptide sequences were compared to the database using Mascot. They matched alpha 1 type II procollagen [Gallus gallus]. According to the m/z of proline, it was clear that the proline residue in the peptide was not hydroxylated and glycosylated. To further assess whether the pC1(II) chains expressed in COS-7 cells formed proper quaternary structures, we also analyzed the assembly of the chains into triple helical molecules by digesting pC1(II) with pepsin. Consistent with the findings of the ESI-MS/MS analysis, we did not detect any pepsin-resistant polypeptides, which would correspond to assembled triple helical pC1(II), on the immunoblot. Altogether, these data demonstrate clearly that the protein expressed in COS-7 cells consists only of non-hydroxylated and non-glycosylated CCII product".

The novel chicken pcDNA-CCOL2A1 therapeutic DNA vaccine prepared by using the full length cDNA encoding a chicken type II collagen CCOL2A1 exhibited significant therapeutic effect in an in vivo rheumatoid arthritis animal model, which effect is very close to that of methotrexate as a pharmaceutical commonly used in clinical treatment of rheumatoid arthritis (see, Vaccines, 2009, 27: 690-700, page 693 paragraph 7 to page 696

paragraph 1), which is confirmed by scoring of swelling degree of osteoarthron in leg in the standard rheumatoid arthritis rat model CIA, radiology tomography, histopathology and anti-type II collagen antibody in serum (see, Vaccines, 2009, 27: 690-700, page 693 paragraph 7 to page 696 paragraph 1).

Withdrawal of the rejection is requested.

Applicants submit the present application is now in condition for allowance. Early notification to this effect is earnestly solicited.

Respectfully submitted,

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